# Indoor fungal composition is geographically patterned and more diverse in temperate zones than in the tropics

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**Fungi are ubiquitous components of indoor human environments, where most contact between humans and microbes occurs. The majority of these organisms apparently play a neutral role, but some are detrimental to human lifestyles and health. Recent studies that used culture-independent sampling methods demonstrated a high diversity of indoor fungi distinct from that of outdoor environments. Others have shown temporal fluctuations of fungal assemblages in human environments and modest correlations with human activity, but global-scale patterns have not been examined, despite the manifest significance of biogeography in other microbial systems. Here we present a global survey of fungi from indoor environments ($n$ = 72), using both taxonomic and phylogeny-informative molecular markers to determine whether global or local indoor factors determine indoor fungal composition. Contrary to common ecological patterns, we show that fungal diversity is significantly higher in temperate zones than in the tropics, with distance from the equator being the best predictor of phylogenetic community similarity. Fungal composition is significantly auto-correlated at the national and hemispheric spatial scales. Remarkably, building function has no significant effect on indoor fungal composition, despite stark contrasts between architecture and materials of some buildings in close proximity. Distribution of individual taxa is significantly range- and latitude-limited compared with a null model of randomized distribution. Our results suggest that factors driving fungal composition are primarily global rather than mediated by building design or function.**

fungal biogeography | indoor mold | latitudinal gradient | phylogenetic community structure | rRNA metagenomics

Indoor environments, where the average person in an industrial nation spends ≈90% of his or her life (1), represent the most important interface between humans and microbes. Examples of well-known fungi include a few human pathogens (2), allergens (3), agents of structural rot (4, 5), and food spoilers (6, 7). Indoor fungi's prominent role in successful litigation around the world contributes to rising costs for various industries and insurance companies (8, 9). Increasingly strict standards for indoor sanitation have resulted in regulatory agencies and private industries seeking to quantify building health. Mould surveys that target the relatively few visibly apparent fungal species or those readily cultivable on artificial media are now standard, and a US Environmental Protection Agency-developed set of real-time PCR probes facilitates their quantification (10). However, the recent rise in fungal infections caused by species formerly considered benign but now seen as causing disease in immunosuppressed humans, and a vastly increased resolution of indoor fungal composition afforded by culture-independent sampling methods (11, 12), force us to reconsider what constitutes a normal indoor environment and the factors that shape it.

Recent efforts to describe the processes shaping indoor and urban fungal composition show temporal effects (13–15) and modest correlations with human activity (16). The existence of global-scale patterns in fungal composition, however, is unexamined, despite evidence of biogeographical patterning in other microbial systems. As with larger organisms, bacterial and archaeal composition is determined by both the contemporary environment and historical processes such as dispersal (17). The relative importance of these factors and the particular environmental variables involved depend on the taxa and habitat sampled.

For indoor fungi whose association with highly mobile humans presents opportunities for global dispersal, we hypothesized that most taxa would be relatively cosmopolitan on a global scale. We also hypothesized that the local indoor environment (as determined by building function, construction material, or circulation system) would play a relatively large role in shaping composition. Finally, as a result of the combination of presumably high dispersal rates between indoor habitats and the highly selective indoor environment, we expected little influence of the *outdoor* environment on fungal composition.

We test these hypotheses (*i*) by examining whether the global distribution patterns of individual taxa show range limitation, (*ii*) by examining whether indoor fungal composition correlates with dispersal limitation or outdoor environment, and (*iii*) by testing an alternative hypothesis that indoor environment determines fungal composition by comparing nearby buildings with different architecture and function.

## Results

**Samples and Processing.** We collected settled dust samples from buildings on six continents ($n$ = 72; Fig. 1 and Table S1) using sterilized collectors attached to vacuum cleaners. We collected settled dust to maximize time integration of fungi accumulating over multiple seasons [rather than the fine-scale temporal partitioning provided by air sampling (18)] and used standardized protocols to collect dust samples pooled from "accessible," "infrequently accessed," and "inaccessible" areas (i.e., behind heavy appliances) to include areas not frequently cleaned. The 61 buildings that were sampled were human dwellings, and the rest were offices, shops, and a church. Extracted DNA was PCR amplified from two loci within the nuclear ribosomal coding cistron: a fragment containing the internal transcribed spacer region 2 (ITS) and a fragment containing the D1 and D2 regions of the large subunit gene (LSU). These were sequenced in multiplex using 454 GS FLX titanium technology (454 Life Sciences). The inclusion of two loci enabled two different types of analysis. The ITS region, an inter-

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Data deposition: Raw sequence data have been deposited in NCBI's sequence read archive under accession nos. SRS010093 and SRS010094 (ITS and LSU sequences, respectively).

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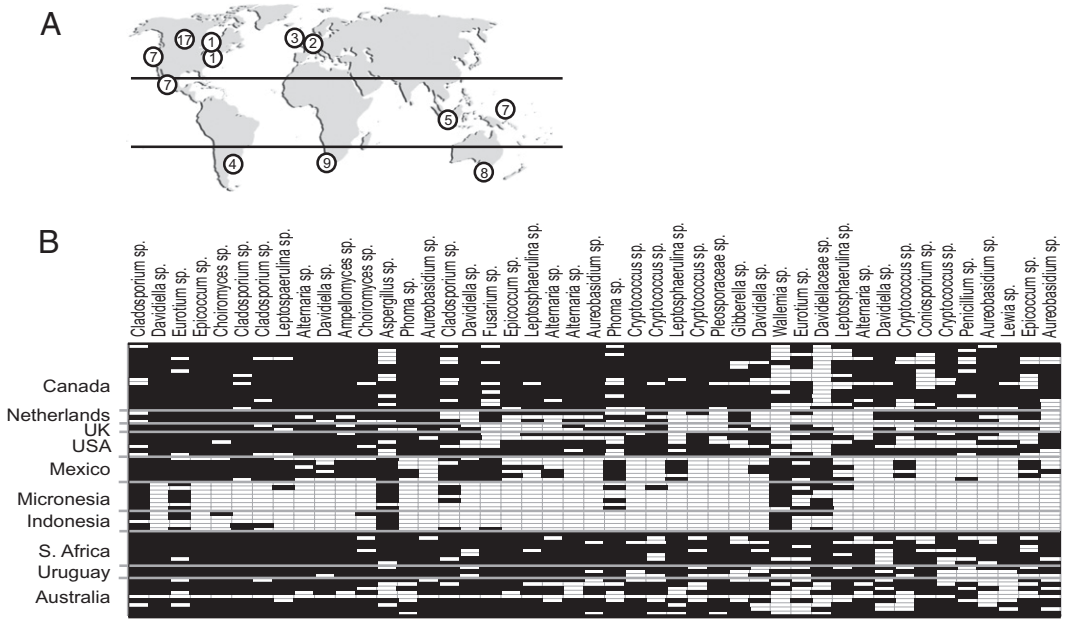


**Fig. 1.** Dust-sampling locations and distributions of the most cosmopolitan OTUs. All samples were collected between December 2008 and March 2009. (*A*) Solid lines indicate the Tropic of Cancer and the Tropic of Capricorn. The number of samples analyzed is noted by the open circles (approximate location). (*B*) The presence of the 45 OTUs found in the most number of samples (from left to right, most cosmopolitan to least cosmopolitan) is indicated by a solid square (samples used in ITS analysis are in rows ordered by latitude). Nomenclature is derived from annotations provided for similar sequences deposited in GenBank. Note the following equivalencies between anamorphic and teleomorphic stages: *Cladosporium* = *Davidiella*, *Aspergillus* = *Eurotium*, and *Gibberella* = *Fusarium*.

genic spacer, is hypervariable and effective for delineating species-level taxonomy, but is unsuitable for multiple sequence alignment and phylogenetic inference at broad levels. Because many environmental sequences derive from unknown or undescribed organisms, operational taxonomic units (OTUs), calculated from pairwise ITS sequence alignment identity, were used instead of species for diversity estimates. Because it is much better represented in public databases such as GenBank, the ITS region was used for taxonomic determinations. The LSU locus, conversely, is phylogenetically informative across fungi, but not divergent enough to distinguish closely related species (19). Here, we use the LSU locus for all comparisons of dust sample phylogenetic composition.

Because 454 sequencing of environmental amplicons can lead to inflated estimates of diversity, partly due to undetected sequence errors (20, 21), we mitigated the effects of these potential errors by removing any sequences containing an ambiguous base call and those where a mis-call occurred in either the priming site or the multiplex tag. The remaining sequences were screened in the CLC Genomics Workbench 2 using the modified Mott trimming algorithm (comparable to Phred score processing), which evaluates sequences on the basis of the 454 quality score. Where base calls did not meet a cumulative error probability threshold of 0.01, they were trimmed from the ends of reads (details of the algorithm and its implementation are available in the CLC Genomics Workbench user manual). Finally, trimmed sequences shorter than 300 bp were removed. The resulting dataset contained 97,557 ITS and 187,668 LSU sequences ranging from 300 to 569 bp (median length 393 bp). For comparative analyses among samples, only those with at least 1,200 LSU reads ($n$ = 62; mean 2,764 ± 2,394 SD reads) or 400 ITS reads ($n$ = 65, mean 1,484 ± 1,539 reads) were included.

To test the effects of artifactual sequence variance on OTU diversity, we included positive controls consisting of five aliquots of one dust sample augmented with equal numbers of fungal spores from individuals of six species along a dilution gradient ($10^6$–10 spores per individual per aliquot; *SI Text*). Spiked dust samples were processed and sequenced as described for other samples. A total of 1,307 sequences homologous to those of the added individuals were recovered, and pairwise distances between sequences within species were calculated at 99.30 ± 0.79%. These sequences clustered into conspecific OTUs at 80–97% ITS sequence identity, whereas 98%, 99%, and 100% identity thresholds resulted in 15, 63, and 701 clusters, respectively. Sequence abundance from individuals added in equal spore counts in our positive control varied from 24 to 633 (Fig. S1). For this reason, we did not use quantitative metrics weighted by sequence abundance to calculate assemblage dissimilarity.

**Geographic Structure of Indoor Fungal Composition.** A minimum evolution phylogenetic tree was constructed from a multiple alignment of all LSU sequences and used to compare sample phylogenetic composition as calculated by the unweighted UniFrac metric (22). This method measures phylogenetic dissimilarity among samples as the proportion of the phylogenetic branch length unique to each. To correct for differences in sample size, 1,200 sequences were randomly selected from each sample, and each analysis was calculated with distance matrices derived from 10 such randomizations (mean statistics and SDs among randomization replicates are reported below).

Phylogenetic similarity of fungal composition correlated most strongly with geography (Table 1 and Fig. 2). Distance from the equator (DFE) was the strongest predictor of assemblage similarity and was colinear with all other measured variables. We used partial Mantel tests to determine the significance of the correlation contributed by each variable in isolation, accounting for the colinearity of the other partial predictors. No variables were significant when DFE was included in partial Mantel models, and therefore we removed DFE from subsequent analyses. With DFE removed, pairwise differences between mean annual rainfall, mean annual temperature, and geographic distance between samples each correlated with sample phylogenetic dissimilarity.

Analysis of similarity (ANOSIM) (23), a nonparametric multivariate analog of ANOVA, indicated that fungal composition of samples in the temperate Northern Hemisphere, temperate

**Table 1. Mantel, partial Mantel, and ANOSIM analyses performed with the phylogenetic composition dissimilarity distance matrix generated using UniFrac**

| Variable of interest | Partial predictor | *R* | SD* | *P* |
|---|---|---|---|---|
| Mantel | | | | |
| DFE[†] | None | 0.572 | 0.008 | **0.001** |
| Distance[‡] | Rain, temperature | 0.138 | 0.008 | **0.005** |
| Rain | Distance, temperature | 0.323 | 0.015 | **0.001** |
| Temperature | Distance, rain | 0.298 | 0.008 | **0.001** |
| ANOSIM | | | | |
| Regional group | None | 0.529 | 0.010 | **0.001** |
| Country | Regional group | 0.614 | 0.010 | **0.001** |
| Building type | Regional group | 0.282 | 0.010 | 0.134 |
| Building type | Country | 0.282 | 0.010 | 0.322 |

Colinear variables accounted for in predictive model are noted as partial predictors. Significant *P* values are shown in bold.
*SD of the *R*-value was calculated among 10 replicates of 1,200 random draws from each sample.
[†]Distance from the equator.
[‡]Geographic distance between samples.

Southern Hemisphere, or the tropics (regional grouping) were significantly more similar to other samples within the same group compared with other samples (Table 1). When we accounted for colinearity of regional grouping, samples within the same country were also significantly more similar to each other than those from different countries. Surprisingly, no significant difference was found between human dwellings and other building types when colinearity of either regional group or country was accounted for, despite stark contrasts between some buildings in close proximity. Some tropical offices and shops, for example, contained tightly sealed windows and air conditioning, whereas some nearby homes in the same location had open ventilation. Thus, the observed compositional variance is consistent with geography and is not an artifact of the types of buildings sampled.

**Phylogenetic and OTU Diversity Gradients.** Contrary to the predominant ecological pattern showing biological diversity increasing with proximity to the equator (24–26), our study showed OTU diversity significantly increased with latitude. (Pearson's product moment $R = 0.37$, $t = 3.111$, d.f. = 63, $P = <0.005$; Fig. 3*D*). Phylogenetic diversity showed a weaker, although significant, pattern ($R = 0.32$, $t = 2.578$, d.f. = 60, $P = 0.01$; Fig. 3*E*).

**Distribution of OTUs and Taxonomic Groups.** OTUs showed distinct patterns of geographic structure. Of 4,473 OTUs defined at 97% ITS sequence identity (Fig. 3*B*), only 31 were found in more than half of the samples. All but 3 of these belonged to the phylum Ascomycota, and 25 of 31 were in the class Dothideomycetes; Fig. 3*A*). The dominant Dothidiomycetes genera found here are also commonly detected indoors in studies using culturing-based methods (6). These genera are mostly composed of species associated with plants and are commonly found on the surface of leaves and/or as saprobes in decaying organic matter. These fungi share several traits, including desiccation-tolerant reproductive structures and melanized spores. Potentially, these characters that aid in dispersal and longevity in outdoor environments confer advantages to fungi on indoor substrates where they commonly grow such as building materials, carpeting, and food.

Other fungi had more limited distributions. We use a randomized permutation-based technique to delimit spatial patterns to reduce bias based on abundance. The mean pairwise geographic distance between samples containing the same OTU was significantly less than expected from a null model of random distribution (Fig. 4*A*), indicating overall spatial limitations to

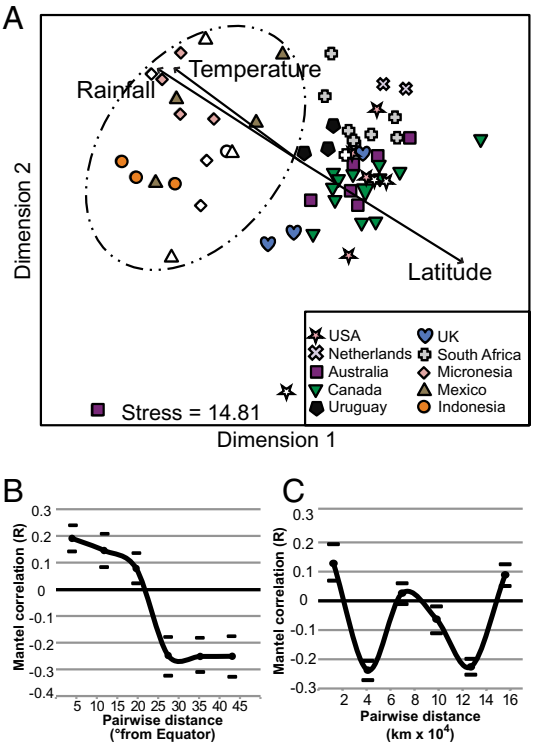


**Fig. 2.** Sample phylogenetic composition similarity is explained by environmental variables. (*A*) Nonmetric multidimensional scaling plot demonstrates goodness-of-fit correlations between the first two dimensions and distance from the equator, mean annual temperature, and mean annual rainfall (all correlations were significant at $P \leq 0.001$). The ordination was calculated using pairwise unweighted UniFrac phylogenetic distance. Open symbols are indoor environments that are not dwellings (e.g., shops, hospitals, a church); the broken line circles tropical samples. Mantel correlograms show assemblage autocorrelation as (*B*) a factor of pairwise differences in latitude and (*C*) pairwise geographic distance between samples. Error bars are 95% confidence intervals. Mean SD of *R*-values from all distance classes among 10 random draws of 1,200 sequences was 0.005 for both latitude and geographic distance measurements.

indoor fungal ranges. This trend is driven by OTUs whose detected ranges are less than 2,000 km, the smallest distance class measured. Of the nonsingleton OTUs, 19.8% were restricted to one of the three regional groups. Conversely, OTUs occurring in both hemispheres were significantly more likely to be found at complementary latitudes (on opposite sides of the equator) than expected (Fig. 4*C*), indicating that the distribution of some dispersal-unlimited species is limited by environmental factors. Similar results were obtained with 90% identity OTU thresholds (Fig. 4*B* and *D*).

We detected indoor fungi that were likely growing within indoor environments as well as those introduced from outdoors. Fungi that were clearly "outdoor" additions to the indoor environment included ecto- and arbuscular-mycorrhizal genera such as *Amanita* and *Glomus*, which are obligate associates of host plants. Genera known only from aquatic environments, such as *Rozella*, were also encountered in indoor dust. All of the obvious outdoor additions were, however, minor components. The presence of mammalian mycobionts such as *Candida albicans*, *Malasezzia* spp., and *Pneumocystis* spp. showed that humans and other mammals also contribute directly to fungi in indoor dust.

## Discussion

This first global survey of indoor fungi demonstrates a previously undetected high diversity of indoor fungi. Although some of the most cosmopolitan taxa in this study (such as *Alternaria*, *Clado-*

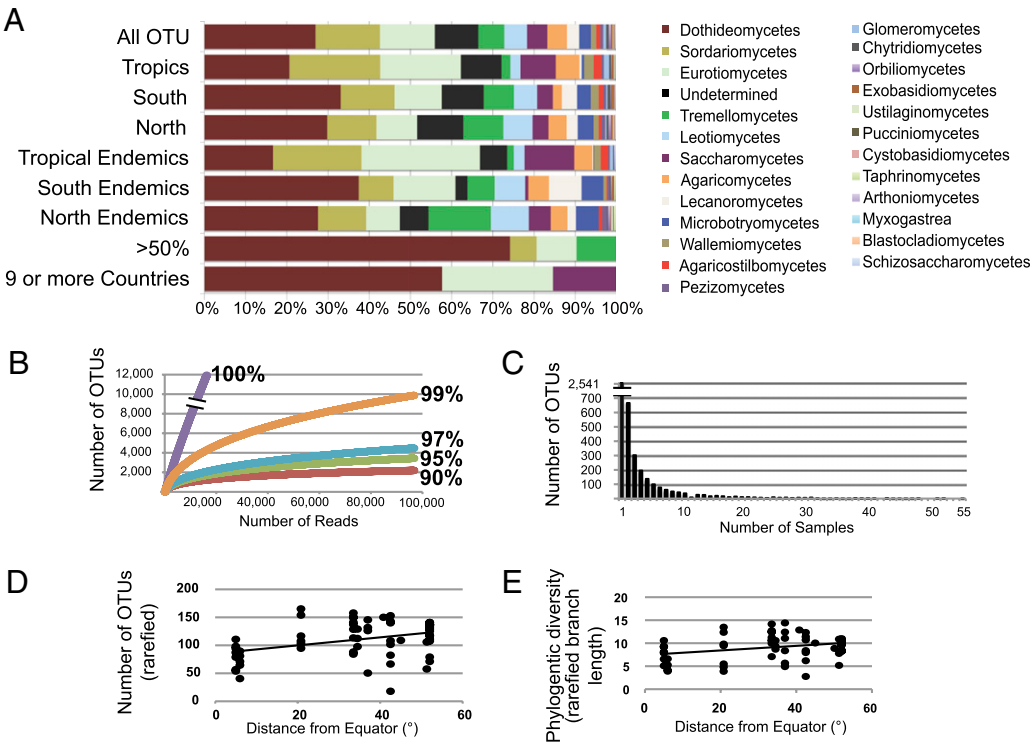


**Fig. 3.** OTU distribution patterns showed little evidence of differentiation at the class level (*A*). The most cosmopolitan OTUs, occurring in more than half of the samples (>50%) or in 9 or 10 of the countries sampled, were dominated by taxa in the class Dothideomycetes. Observed OTU rarefactions (*B*) were calculated at ITS percentage identity thresholds labeled as 100%, 99%, 97%, 95%, and 90%. A 100% identity rarefaction curve is not contained in the figure, but is calculated to be 54,815. OTU frequency distribution (*C*) has a long tail with 2,541 (56%) OTUs occurring in a single sample only. Within-sample OTU richness (*D*; Pearson's product moment, $R = 0.37$, $t = 3.57$, df = 63, $P = <0.001$; rarefied to 400 sequences per sample) and phylogenetic diversity, measured as cumulative branch length (*E*; $R = 0.32$, $t = 2.58$, df = 60, $P = 0.01$; rarefied to 1,200 sequences per sample), were positively correlated with distance from the equator.

*sporium*, *Epicoccum*) are also common in culture-based studies, fast-growing Eurotiomycetes (i.e., *Penicillium*, *Aspergillus*, *Paecilomyces*, and their teleomorphs), Sordariomycetes (*Fusarium*, *Stachybotrys*), and Zygomycota (Mucorales) tend to dominate on cultivation media. The significance of our results is not merely the high taxonomic richness of fungi detected indoors, but also the phylogenetic diversity that includes every major lineage of fungi. These results force us to broaden our outlook on measuring and, perhaps, regulating normal indoor environments.

Microbial biogeographical theory (17) suggests that biogeographic patterns of species may result from historical contingencies including dispersal limitation, contemporary environmental selection, or some combination of the above. Our results do not support any one unified process over the others and instead suggest that different OTUs are subject to different limitations. We found many OTUs limited to ranges less than 2,000 km (suggesting historical contingencies or contemporary environmental selection), others with wide ranges but limited by latitude (suggesting contemporary environmental selection but not historical contingencies), and others with clearly "cosmopolitan" distributions suggesting neither historical contingencies nor contemporary environmental selection as constraints. Determining whether fungal distribution patterns correlate with, e.g., substrate preference, propagule size, trophic status, etc., may offer a starting point for future hypothesis testing of factors driving biogeography.

The degree to which these biogeographic patterns detected in indoor environments apply to outdoor fungal systems remains untested, although global, disjunct distributions occur in other microbial taxa, such as psychrophilic cyanobacteria species in polar regions (27). We might expect indoor fungi to be dispersed by human-mediated global travel and commerce, whereas outdoor fungi in natural systems are more limited by natural dispersal mechanisms such as wind or splash. Therefore, the strong geographic patterns that we find in indoor fungal OTUs point very strongly toward environmental selection rather than dispersal limitation.

We find that latitude has the greatest influence on indoor fungal compositional variation. This likely reflects correlations with local outdoor environmental variables such as rainfall and temperature (and other variables not measured here). These results are consistent with those from other studies showing strong correlations between environmental conditions and microbial composition. A global study of Acidobacteria communities in soil, for example, found that community OTU (28) and phylogenetic (29) similarity was predicted best by pH, whereas other studies found strong correlations with environmental parameters such as salinity and nutrient gradients (30–32). Our study found that distance between samples explains only a minor component of fungal composition dissimilarity. If dispersal limitation were a more critical factor in shaping indoor fungal composition, we might expect a stronger correlation as species turnover results in dissimilarity by distance patterns (33, 34). In our case, however, where shared phylogenetic branch length rather than shared species was used to calculate compositional similarity, dissimilarity by distance may not be a good indicator of dispersal limitation per se.

One of the most surprising results of our analysis of OTU distributions is a higher diversity in temperate compared with tropical zones. Although recent work proposes that similar trends may exist among ectomycorrhizal fungi (35, 36), this pattern runs contrary to almost all measured patterns of global biodiversity, including those of other microbial systems. Pianka's seminal review of latitudinal diversity gradients in macroflora/fauna (24) suggests numerous processes that potentially drive a lower di-

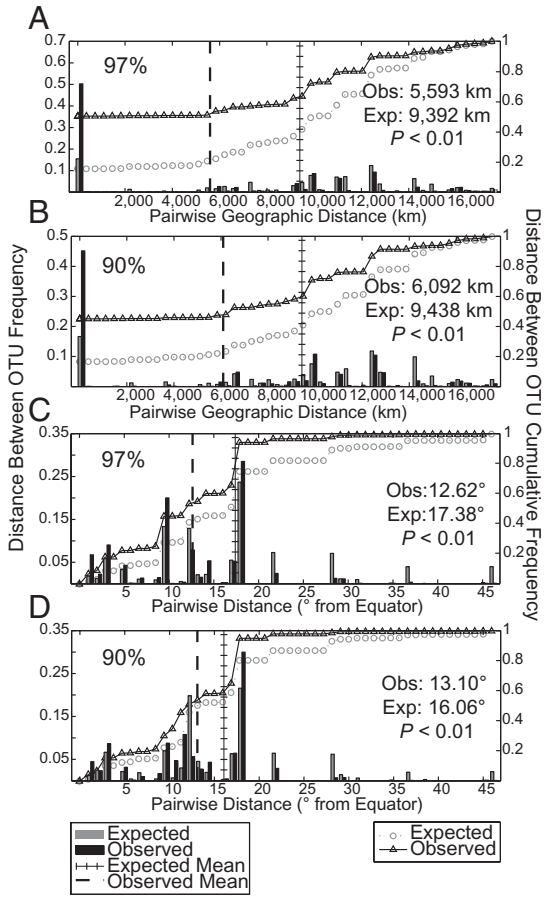


**Fig. 4.** Mean pairwise distance between samples sharing nonsingleton OTUs is significantly less than would be expected under a null model of random distribution when OTUs are defined at 97% sequence identity (*A*; $n$ = 1,932;) and at 90% sequence identity (*B*; $n$ = 1,258). OTUs shared among buildings located within 2,000 km appear to drive this geographic structure, indicating relatively small ranges of some OTUs. Pairwise comparisons of samples from opposite sides of the equator (within-hemisphere pairwise comparisons excluded) showed that OTUs were more likely to be shared among samples taken from similar distances from the equator than would be expected under a null model of random distribution with OTUs defined at 97% sequence identity (*C*) and 90% sequence identity (*D*). Histograms represent the frequency of mean pairwise distance between locations of each OTU in distance classes. Expected means and significance value were calculated with 100 randomized permutations of the dataset constrained by sequence abundance at each location. Vertical lines are the mean of all individual OTU calculations. Triangles and circles are the cumulative frequency of OTUs at increasing distance. *P* value is the likelihood that the observed mean is greater than the expected.

versity in temperate zones, including diminished spatial heterogeneity, decrease in competition and predation, a less stable climate, and lower productivity. How these factors may apply to microbial communities is unclear, but plausible contradictory hypotheses would not be difficult to devise. Because our samples were time-integrated, we may have measured the diversity of seasonally admixed fungal composition that may not all be metabolically active at the same time. Temporal heterogeneity associated with more pronounced seasonality in temperate zones may be one difference between microbes with short reproductive cycles and their longer-lived, larger-bodied counterparts.

Although correlations between human socio-economics and latitude are well documented (37), our results indicate that factors such as building materials, content, or use have no significant effect on fungal composition. Fungal composition in vastly different types of buildings was most similar to other structures in close proximity. Despite humankind's best efforts to homogenize indoor climates, local environmental selection outside the buildings themselves appears to be the strongest determinant of indoor fungal composition. Therefore, we suggest that indoor habitats should not be thought of as microcosms isolated by weatherstripping and HVAC filters, but rather as compositional subsets of a larger biome.

## Materials and Methods

**Dust Collection.** Protocols were adapted and modified from the US Environmental Protection Agency (www.epa.gov/wtc/panel/pdfs/6-att-4a-indoor_dust_sampling_protocols_061705.pdf). To maximize time integration of settled dust, samples were collected from (*i*) accessible, (*ii*) infrequently accessed, and (*iii*) inaccessible areas in replication numbers scaled to unit size. Dustream collectors (Indoor Biotechnologies) were sterilized before use by soaking in 10% bleach and dried under UV radiation. These samplers were attached to domestic vacuum cleaners for collection. Samples were filtered through a 2-mm sieve and refrigerated at 4 °C until further processing. Samples collected from Regina, Saskatchewan, Canada, were collected by Health Canada in conjunction with a cohort study of asthmatic and control households, following similar collection methods. The sample from Stittsville, ON, Canada, was extracted from the storage container of a domestic central vacuum system. All samples were collected between December 2008 and March 2009.

**DNA Extraction.** Duplicate, 100-mg sample aliquots were processed using previously published methods (38) with the following modifications: tubes were beat on a Biospec Mini 8 bead mill (Biospec) set to homogenize for 1 min, and DNA was eluted with 50 μL of 1× TE (10 mM Tris pH 8, 1 mM EDTA). DNA concentration was quantified on a NanoDrop spectrophotometer (ThermoScientific).

**PCR Amplification.** Approximately 1.25 ng of each duplicate DNA extraction was added to a PCR mixture containing 1.2 units of HotStarTaq polymerase (Qiagen), 1× PCR buffer supplied by the manufacturer, 200 μM of each DNTP, 0.5 μM of each primer, and $H_2O$ to a final concentration of 25 μL. ITS amplification used concatemers (Table S2) containing either the primer pair ITS1f and ITS4 (39) or the primer pair LROR_F (excluding animals; Table S3) and LR5-F [(40) excluding plants], with LROR_F as the forward primer (LSU). Following an initial denaturation at 95 °C for 10 min, PCR was cycled 34 times at 95 °C for 1 min, 51 °C (ITS) or 54 °C (LSU) for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 7 min. Periodic negative controls were run on samplers for every 24 DNA extractions and on every PCR.

PCR products were cleaned using the QIAquick PCR purification kit (Qiagen) following the manufacturer's instructions, quantified using a Qubit Fluorometer (Invitrogen) pooled by locus into equimolar concentrations, and sequenced using approximately five-eighths of a 454 Titanium sequencing run. LSU and ITS amplicons were run in separate regions of a gasketed plate.

**Sequence Processing and Analyses.** Reads were sorted by multiplex tags using the Ribosomal Database Project (RDP) pyrosequencing pipeline (41). ITS sequences were clustered into OTUs using the program CD-HIT-EST (42) using the "accurate but slow" iteration algorithm. Resulting clusters were input into the software package MOTHUR (43) for rarefaction analyses. OTUs clustered at 97% were compared using BlastN against all identified fungal sequences in GenBank and determined to the last common ancestor (LCA) using the program MEGAN [(44); LCA parameters: minimum support—1, minimum score—200, score/length ratio—1.97, top percentage—1] and cross-referenced to class-level designation with the Dictionary of Fungi (45) as an authority in an SQL database.

LSU sequences were aligned using MAFFT [(46); parameters: retree 1, maxiterate 0, nofft, parttree). Alignment columns containing more than 80% gaps were trimmed using trimAL (47), and FastTree (48) was used for phylogenetic tree construction. The command-line version of the unweighted UniFrac algorithm was used for phylogenetic diversity rarefaction, and the phylogenetic dissimilarity matrix was used in multivariate analyses. All multivariate statistics were calculated using the Vegan (49) and Ecodist (50) packages in the R programming environment (51). Confidence intervals for Mantel and ANOSIM analyses were calculated with 999 resamples. Mean geographic distance between OTU locations was calculated using a modification of the program SAShA (52). Null distributions and significance values were calculated by randomizing OTU locations while maintaining distance classes and the number of reads per sample constant. We ran SAShA with 100 random permutations in MATLAB v. R2008b (MathWorks).

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